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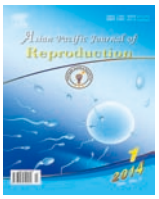

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Aphrodisiac and phytochemical studies of *Cocculus hirsutus* extracts in albino rats

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ABSTRACT

Objective: To investigate the *in vivo* aphrodisiac activity of various extracts obtained from aerial parts *Cocculus hirsutus* (*C. hirsutus*). **Methods:** We evaluated whether oral administration of *C. hirsutus* has spermatogenic activity in male rats. Petroleum ether, chloroform and alcohol extract of aerial parts (stem and leaf) of *C. hirsutus* were administered at the dose level of 25 mg/100 g body weight to adult male albino rats for 30 days. **Results:** The above said extracts have stimulated the spermatogenic activity and accessory reproductive organs performance in albino rats. Out of the three extracts administered, alcohol extract showed highly stimulant spermatogenic effects in mature male albino rats. Alcohol extract showed potent androgenic activity when treated in immature Albino rats. Males treated with the extract displayed more frequent and vigorous anogenital sniffing and mounting as compared to untreated animals. The increased spermatogenesis in extract treated groups was confirmed by change in histoarchitecture as evidenced by increase in number of spermatogonia, spermatocyte, spermatids and caudal spermatozoa. After subjecting to preliminary phytochemical screening, the alcohol extract showed positive tests for steroids, saponins, oils and fats, phenolic compounds and tannins. **Conclusion:** *C. hirsutus* exhibited remarkable increase in spermatogenic activity. These findings support the folk use of this plant as an aphrodisiac.

1. Introduction

Perpetuation of one's race is the dogma of all living organisms. All living organisms strive to achieve this through the process of reproduction, which is the vital process that enables a species to represent itself in the following generation in the form of its offspring. Different contraceptive methods are in practice for family planning (i.e. for population control). At the same time there are couples who are facing the problems of infertility. Infertility is the diminished ability or the inability to conceive and have offspring. Statistics reveal that more than 2 million married couples are now experiencing problems with infertility. Approximately 6 million women between the ages of 16 and 45 have infertility issues and about 30% of cases are found in the man alone. But infertility is not always a women's problem. Infertility are due to the women (female factors) or man (male factors) or caused by a mixture of male and female factors or by unknown factors.

There are different types of assisted reproductive technologies (ART) that are used to treat infertility. These treatments are highly effective when it comes to increasing the chances of conception, but are very expensive and are often associated with a number of physical and emotional side effects. About 30%-50% of problems in infertile couples are due to male infertility. Present day's people are turning to herbal remedies to improve this infertility problem as they are easily approachable to common man. Researches are carried out to find out the plant products that can be used to treat this kind of infertility problems. Several plant extracts have long been used to treat problems with fertility. In fact, evidence of the use of herbal extracts for male and female fertility dates all the way back to 200 A.D.

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Cocculus hirsutus (*C. hirsutus*) belongs to the family Menispermaceae, a perennial climber that can form a dense cover on top of other plants mainly found in tropical and subtropical climatic conditions[1]. In India it is found almost throughout in open habits and dry localities including Karnataka, Uttar Pradesh, Gujarat, Orrisa, Rajasthan, Tamil Nadu, Bihar, West Bengal, Maharastra. It has a special potency as a detoxifier. The leaves are useful in gonorrhoea, cough, ophthalmia, cephalalgia, neuralgia and also used to treat skin infections and itchy skin including rheumatism[2]. In Rajasthan (India), the cooked leaves are eaten to treat night blindness. The juice of leaves is used externally as a cooling and smoothing agent in eczema, impetigo[3]. Aerial parts of the plant reported to be used as a diuretic, laxative[4] and root extract showed analgesic and anti-inflammatory effect[5].

Recent studies have shown the antidiabetic and spermatogenic activity of *C. hirsutus* in rats[6]. Therefore, it is our interest to process this plant for systematic study on its stimulatory effect on reproduction in male rats.

2. Materials and methods

2.1. Plant material

The plant material was collected from local farmers in and around Gulbarga (North Karnataka, India) during February to March 2009. The aerial parts were shade dried inside the laboratory and then subjected to soxhlet extraction.

2.2. Preparation of plant extract

The dried aerial parts (*i.e.*, stem and leaves) were finely powdered and subjected to soxhlet extraction successively and separately from non polar to polar solvent i.e., petroleum ether, chloroform and alcohol. The extracts were concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (50 ℃-60 ℃) to obtain the crude extract. The extract were preserved in a refrigerator at 4 ℃ and diluted as required dosage.

2.3. Phytochemical screening

The petroleum ether, chloroform and alcohol extracts of *C. hirsutus* were subjected to phytochemical tests as described by Kokate[7], Harborne[8] and Farnsworth[9] to determine the presence (qualitatively) or absence of various chemical constituents.

2.4. Experimental animals

Sexually matured, healthy, male albino rats of Wister strain (*Rattus norvegicus*), weighing about 170-190 g were used for the experiments. The rats were given pelleted feed and tap water *ad libitum*. The temperature was maintained at (25 ± 20) ℃. All the extracts were prepared in Tween-80(1%), suspended in distilled water and administered orally to the animals with the help of intragastric catheter at desired dose. The control animals received an equivalent amount of vehicle only.

2.5. Aphrodisiac activity of extracts in male albino rats

Adult virgin male albino rats of Wistar strain were used for the experimentation. The animals were divided into 4 groups of 6 animals each and treated as follows.

Group I: Control, received 0.2 mL tween-80 (1%); Group II: Received 25 mg/100 g body weight of petroleum ether extract in 0.2 mL tween-80 (1%); Group III: Received 25 mg/100 g body weight of chloroform extract in 0.2 mL tween-80 (1%); Group IV: Received 25 mg/100 g body weight of alcohol extract in 0.2 mL tween-80 (1%).

All the above treatments were given orally for 30 days. On the 31st day all the rats were scarified and the testis, epididymis, vas deference, seminal vesicle, prostate were dissected out, surrounding blood vessels and tissues were removed and blotted free of blood and mucous. The tissues were weighed quickly to the nearest mg on an electronic balance. The organ weights were expressed in terms of 100 g body weight.

2.6. Biochemical estimations

The testis and epididymis obtained from one side from each animal was used for the estimation of protein [10], glycogen[11], cholesterol[12].

2.7. Histological studies

The testis and cauda epididymis from the other side was fixed in Bouine's fluid, embedded in paraffin, sectioned at 5 μm thickness and stained in haematoxylin and eosin and processed for histological studies[13].

2.8. Micrometry

Micrometric measurements like diameter of testis, seminiferous tubules were made from randomly selected 20 sections of 6 animals of each group which appeared round in cross section[14]. The micrometric studies were made by using stage and ocular micrometer.

2.9. Counting of spermatogenic elements

Randomly chosen 20 round sections of testis from each group and observed under the microscope. The spermatogenic elements like spermatogonia, spermatocytes and spermatids were counted from each section and then average of each spermatogenic elements was calculated[15].

2.10. Sperm count

The sperm count in the cauda epididymis was made by the method described by Wyrobck and Bruce[16].

2.11. Androgenic activity of C. hirsutus in immature male Albino rats

This experiment was designed to know the androgenic/antiandrogenic effect of alcohol extract of *C. hirsutus* aerial parts. The immature Albino rats of Wistar strain of 25-30 days old, weighing between 30-40 g were used for the experimentation. The animals were divided into 4 groups containing 6 rats in each group.

Group I: Control, received 0.2 mL tween-80 (1%); Group II: Received 10 μg testosterone /rat/day in 0.1 mL olive oil; Group III: Received 25 mg/100 g body weight of alcohol extract in 0.2 mL tween-80 (1%); Group IV: Received 25mg/100gm body weight of alcohol extract in 0.2 mL tween-80 (1%) + 10 μg testosterone/rat/day in olive oil.

2.12. Statistical analysis

The results were expressed as mean ± SE of the 6 rats per group. All the values were statistically analysed by using *Students 't'* test. The values were judged almost significant if $P<0.05$, significant if $P<0.01$ and highly significant if $P<0.001$.

3. Results

Petroleum ether showed positive test for oils and fats. Chloroform extract showed positive tests for alkaloids, steroids, glycosides, saponins, oil and fats and phenolic compound and Tannins. Alcohol extract showed positive test for steroids, saponins, oils and fats, phenolic compounds and tannins (Table 1).

The body weight has increased in all the experimental animal groups. This increase is 10.98% in control rats whereas it is 21.47%, 14.21% and 14.06% respectively in the rats treated with petroleum ether, chloroform and alcohol extracts. Six rats were maintained in all the groups. No mortality is observed in the rats either in saline treated control or different extracts of *C. hirsutus* treated experimental groups (Table 2).

The weight of testis is increased highly significantly ($P<0.001$) with the administration of petroleum ether, chloroform and alcohol extracts when compared with that of control (Table 3). The diameter of testis is increased highly significantly ($P<0.001$) with all the extract treated groups when compared to that of control group. The diameter of seminiferous tubules showed parallel increase ($P<0.001$) in comparison with that of control (Table 4 and Figure 1-4).

Table 1
Phytochemical screening of crude petroleum ether, chloroform and alcohol extracts of aerial parts of *C. hirsutus*.

Tests		Petroleum ether extract	Chloroform extract	Alcohol extract
1. Alkaloids	(a) Mayer's test	−	+	−
	(b) Wagner's test	−	+	−
2. Steroids	(a) Solkowaski's test	−	+	+
	(b) Libermann-Burchard's test	−	+	+
3. Glycosides	(a) Sulphuric acid test	−	+	−
	(b) Molisch's test	−	+	−
4. Amino acids and proteins	(a) Ninhydrin test	−	−	−
	(b) Million's test	−	−	−
5. Saponins	(a) Aqueous test	−	+	+
6. Flavones	(a) Aqueous NaOH test	−	−	−
	(b) H_2SO_4 test	−	−	−
7. Anthocyanins	(a) Aqueous NaOH test	−	−	−
	(b) H_2SO_4 test	−	−	−
8. Oils and fats	(a) Spot test	+	+	+
	(b) Saponification test	+	+	+
9. Phenolic compounds and tannins	(a) $FeCl_3$ test	−	+	+
	(b) Lead acetate test	−	+	+

'+' = Positive test; '−'= Negative test

Table 2

Effect of different extracts of *C. hirsutus* on the body weight and mortality in albino rats.

Treatment	Dose (mg/100 g body weight)	Initial body weight (g)	Final body weight (g)	Percentage change (%)	Mortality alive/total (%)
Control	Tween-80 (1%)	173.00±0.54	192.00±1.51	10.98	6/6 (100%)
Petroleum ether	25	177.00±0.89	215.00±1.87	21.47	6/6 (100%)
Chloroform	25	190.00±1.41	217.00±1.81	14.21	6/6 (100%)
Alcohol	25	192.00±0.55	219.00±3.41	14.06	6/6 (100%)

M±SE=Mean ±Standard error

Table 3

Gravimetric changes in the testis and accessory organs due to oral administration of different extracts of *C. hirsutus* in albino rats.

Treatment	Testis	Epididymis	Vas deferens	Seminal vesicle	Prostate gland
Control	566.07±9.91	292.60±5.55	77.33±1.02	366.93±10.69	150.88±0.23
Petroleum ether	930.47±34.67**	371.38±2.34**	80.95±1.11*	431.17±11.24**	287.58±3.23**
Chloroform	1024.86±12.64**	359.79±3.68**	95.73±0.66**	478.29±2.36**	235.78±1.98**
Alcohol	1084.82±7.36**	379.85±2.38**	97.76±1.62**	439.50±6.75**	284.64±3.10**

Dose: 25 mg/100g body weight; Duration: 30 days; Organ weight: mg/100 g body weight; M ± S = Mean ± Standard Error. Six animals were maintained in each group. *P<0.05; **P<0.01; ***P<0.001 when compared with control.

Table 4

Histometric and biochemical changes in the testis due to oral administration of various extracts of *C. hirsutus* in albino rats.

Treatment	Weight of testis (mg)	Diameter of testis (μ m)	Diameter of seminiferous tubules (μ m)	Protein (μ g/mg)	Cholesterol (μ g/mg)	Glycogen (μ g/mg)
Control	566.07±9.91	2 146.32±10.34	166.20±4.10	14.80±0.8	1.04±0.07	0.37±0.05
Petroleum ether	930.47±34.67**	2 548.23±6.39**	309.91±5.75**	20.72±1.68**	0.94±0.06*	0.42±0.01*
Chloroform	1 024.86±12.64**	2 082.26±4.13**	318.15±3.96**	20.0±1.05**	0.90±0.02*	0.41±0.04*
Alcohol	1 084.82±7.36**	2 960.49±5.59**	335.28±6.91**	26.16±1.38**	0.82±0.11**	0.47±0.06**

Dose: 25 mg/100 g body weight; Duration: 30 days; M ± SE = Mean ± Standard Error. Six animals were maintained in each group; *P<0.05; **P<0.01; ***P<0.001 when compared with control.

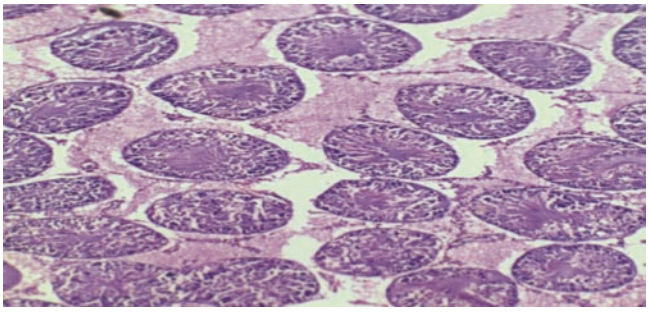

Figure 1. Control rat showing normal seminiferous tubules with normal spermatogenesis.

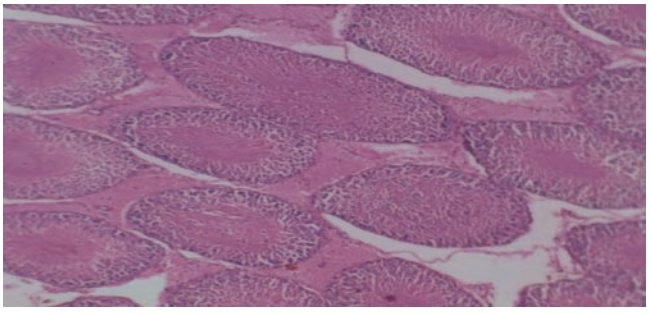

Figure 2. Petroleum ether extract treated rat showing all types of spermatogenic elements and spermatozoa in the lumen.

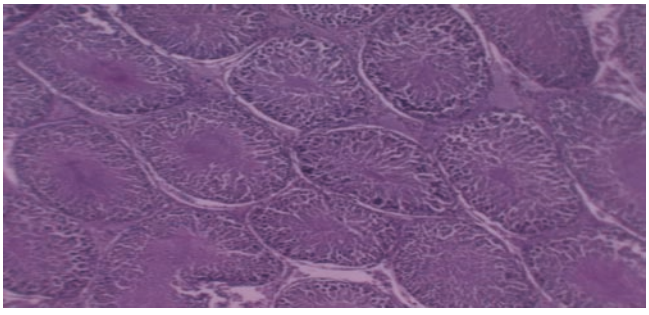

Figure 3. Chloroform extract treated rat showing increased number of spermatogonia, spermatocytes and spermatids and more number of spermatozoa in lumen.

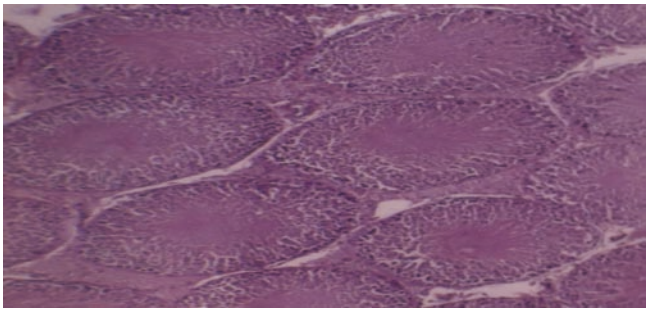

Figure 4. Alcohol extract treated rats showing increase in the size of seminiferous tubules, significant increase in the spermatogonia, spermatocytes and spermatids and presence of large number of spermatozoa.

The protein content of the testis is increased significantly (P<0.001) in all the groups treated with petroleum ether and chloroform extract. This increase is highly significant (P<0.001) in the group which received alcohol extract. The cholesterol content of the testis in the rats that received petroleum ether and chloroform extracts is decreased less significant (P<0.05), whereas it is significant (P<0.01) with the treatment of alcohol extract. The glycogen content of the testis shows increase due to the administration of all the extracts of *C. hirsutus*. It is significant (P<0.01) with alcohol extract (Table 4).

The number of spermatogonia is increased in all the treated groups, but it is significant (P<0.01) with that of alcohol extract. The number of spermatocytes is increased with the treatment of petroleum ether extract, but this increase is significant (P<0.01) with chloroform extract and highly significant (P<0.001) with that of alcohol extract. The numbers of spermatids increased almost significantly in the groups that received chloroform and alcohol extracts (Figure 1-4). The cauda epididymal sperm count was made by using haemocytometer. The number of caudal spermatozoa is increased significantly (P<0.01) with all the extracts of *C. hirsutus* (Table 5 and Figure 5-8).

Table 5

Effects of oral administration of various extracts of *C. hirsutus* on the spermatogenic elements of the testis in albino rats.

Treatment	Spermatogonia	Spermatocytes	Spermatids	Sperm count millions/mg cauda
Control	81.40±2.60	121.33±6.56	173.46±5.62	45.47±3.76
Petroleum ether	84.36±3.20	135.81±8.93	189.12±3.32*	61.07±2.22**
Chloroform	86.79±5.62	153.16±5.61**	219.43±4.69***	64.80±0.97**
Alcohol	95.31±2.30**	213.49±4.91***	249.56±7.42***	65.18±3.36**

Dose: 25 mg/100 g body weight; Duration: 30 days; M ± S = Mean ± Standard error. Six animals were maintained in each group; $^{*}P$<0.05; $^{**}P$<0.01; $^{***}P$<0.001 when compared with control.

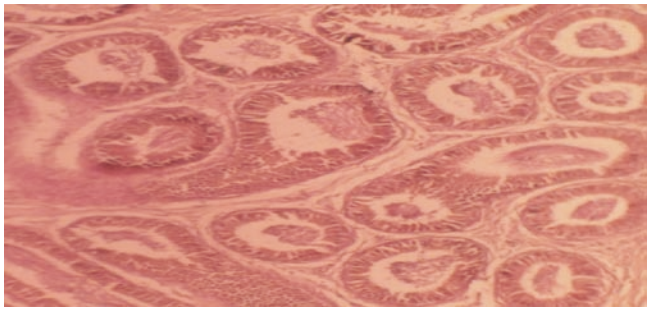

Figure 5. Control rat showing ductules with normal testicular secretions and spermatozoa in the lumen.

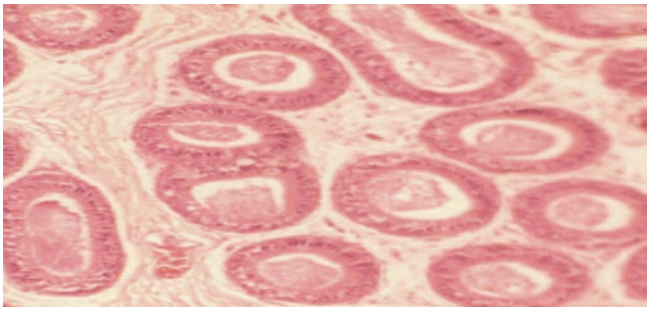

Figure 6. Petroleum ether extract treated rats showing increase in the size of cauda filled with almost significant number of spermatozoa.

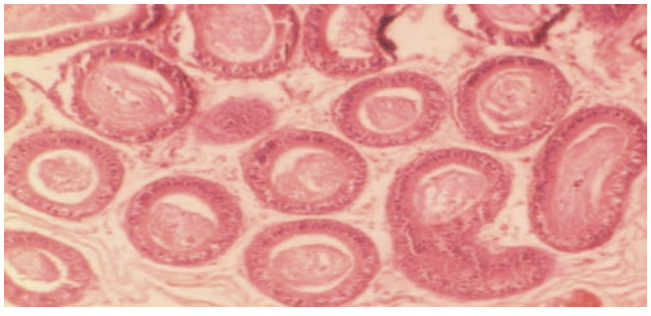

Figure 7. Chloroform extract treated rats showing increase in the size of cauda filled with high number of spermatozoa.

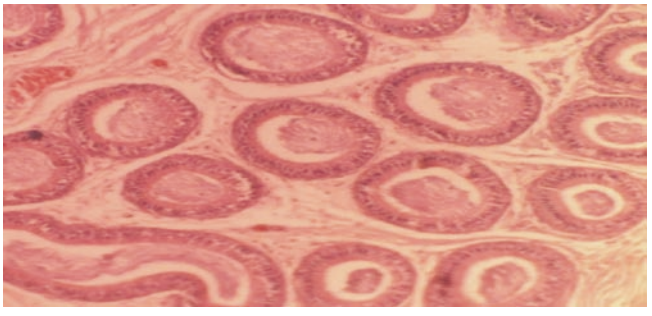

Figure 8. Alcohol extract treated rats showing highly increase in the size of cauda filled with highly significant number of spermatozoa.

The weight of epididymis is increased in all the groups treated with the extracts of *C. hirsutus*. The weight of vas deference is increased almost significantly ($P<0.05$) with the treatment of petroleum ether and highly significantly ($P<0.001$) with the treatment of chloroform and alcohol extract. The seminal vesicle shows significant ($P<0.01$) increase with the treatment of petroleum ether extract and highly signicant with that of chloroform and alcohol extracts. Highly significant ($P<0.001$) increase in the weight of prostate gland is seen with the treatment of all the three extract of *C. hirsutus* to Albino rats (Table 3).

The protein content of epididymis is increased highly significantly ($P<0.001$) with all the treatments of petroleum ether, chloroform and alcohol extracts. Almost significant ($P<0.05$) decrease is observed in cholesterol content of the epididymis. Similarly a significant ($P<0.01$) increase in glycogen content is observed in all the experimental groups treated with different extracts of *C. hirsutus* when compared to that of control group (Table 6).

The androgenic/antiandrogenic activity is carried out in immature 25-30 days rats by the administration of alcohol extract which is the most effective extract amongst the tested extracts. The weight of the testis is increased significantly ($P<0.01$) with the treatment of testosterone. The testosterone administration has increased all the accessory organs like epididymis, vas deferens, seminal vesicle and prostate gland highly significantly.

Alcohol extract administration to the immature male rats has increased the weight of testis significantly ($P<0.01$). But all the accessory organs show highly significant increase ($P<0.001$) with the administration of alcohol extracts of *C. hirsutus*. This result indicates clearly the androgenic effect of alcohol extract.

When the alcohol extract and testosterone extract were administered conjointly, this increase is more than that of separate administration of testosterone or alcohol extract. Therefore the result of this experiment indicates not only that, alcohol extract of *C. hirsutus* is having androgenic effect but this androgenic effect is synergistic with that of testosterone (Table 7).

Table 6

Biochemical changes in epididymis due to oral administration of various extracts of *C. hirsutus* in albino rats.

Treatment	Weight of epididymis (mg)	Protein (μ g/mg)	Cholesterol (μ g/mg)	Glycogen (μ g/mg)
Control	292.60±5.55	13.56±0.08	0.92±0.28	0.11±0.03
Petroleum ether	371.38±2.34***	19.63±0.63***	0.80±0.11*	0.15±0.02**
Chloroform	359.79±3.68***	18.87±0.79***	0.84±0.04*	0.16±0.10**
Alcohol	379.85±2.38***	21.53±0.39***	0.81±0.10*	0.17±0.10**

Dose: 25 mg/100 g body weight; Duration: 30 days; M ± S = Mean ± Standard Error. Six animals were maintained in each group; *$P<0.05$; **$P<0.01$; ***$P<0.001$ when compared with control.

Table 7

Effects of testosterone, alcohol extract and their combinations administered intraperitoneally on the weights of testis and accessory sex organs in immature male Albino rats.

Treatment	Testis	Epididymis	Vas deferens	Seminal vesicle	Prostate gland
Control	863.55±20.00	77.98±0.98	27.51±2.09	18.03±0.25	18.58±0.28
Testosterone	899.29±26.92**	130.13±4.80***	43.91±1.79***	90.94±4.86***	37.50±1.36***
Alcohol extract	952.03±31.09**	99.78±4.14***	46.54±1.69***	40.16±1.26***	28.54±0.40***
Testosterone + alcohol extract	983.99±25.90**	172.79±2.79***	62.25±1.02***	178.18±5.23***	55.46±3.94***

Duration: 7 days; Organ weight: mg/100 g body weight; M ± S = Mean ± Standard error. Six animals were maintained in each group; *$P<0.05$; **$P<0.01$; ***$P<0.001$ when compared with control.

4. Discussion

In the present study administration of *C. hirsutus* extracts have stimulated the activity of testis and accessory organs. Out of three extracts administered alcohol extract is proved to be highly stimulant, chloroform extract is medium stimulant and petroleum ether is less stimulant in increasing the weight of testis and male reproductive accessory organs. There is also a progress in spermatogenesis as seen in the increase of spermatogenic elements in the testis and sperm count in cauda epididymis which may be due to the higher availability of pituitary FSH, as FSH is known to stimulate the spermatogenesis[17-20]. Both FSH and LH/ICSH are necessary for meiosis and formation of spermatids[21-24]. The observed increase in the number of spermatogonia, spermatocytes and spermatids and in cauda epididymal spermatozoa may be attributed due to the increased availability of FSH and LH in chronic *C. hirsutus* extracts treated rats.

It is known that sperm production cannot proceed optimally to completion without continuous androgen supply[25]. However the incidence of high sperm count in experimental rat treated with *C. hirsutus* extracts may be due to higher availability of androgens. Testis is an androgen

dependent organ. The androgen synthesis in the testis is dependent on pituitary FSH and LH. The increased weight in the accessory organ in treated rats proves the extract may stimulate the FSH and LH release and testosterone production. The study regarding the androgenic activity of *C. hirsutus* alcohol extract clearly indicates the androgenic activity of the *C. hirsutus*.

The high level of protein observed in the testis and accessory organs indicates the enhancement of testicular growth as FSH is essential for protein synthesis in gonads and accessory organs[26]. Increase in the glycogen concentration is the indication of increase in testosterone synthesis as the glycogen level is found to be indirectly proportional to steroid hormone production[27]. The cholesterol which is a precursor for androgen synthesis might have been utilized for testosterone synthesis in *C. hirsutus* treated rats.

The phytochemical screening indicates that petroleum either extracts contains oils and fats, chloroform extract contains alkaloids, steroids, glycosides, saponins, oils and fats, phenolic compounds and tannins; alcohol extract contains steroids, saponins, oils and fats, phenolic compounds and tannins. Based on this study it may be concluded that maximum spermatogenic stimulant activity and androgonic activity of alcohol extract of *C. hirsutus* may be due to the absence of alkaloid.

As the body weight of the all the extract treated rat is increased and no significant change in the food take and behavioral pattern is observed. It may be concluded that *C. hirsutus* extracts may have not side effects on experimental animals.

Conflict of interest statement

We declare that we have I conflict of interest.

Acknowledgements

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